

CLAIMS

1. A method for detecting an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:

- (a) providing a nucleic acid sequence encoding said mRNA interferase or a functional fragment thereof;
- (b) expressing said nucleic acid sequence;
- (c) incubating the expressed nucleic acid sequence of step (b) with an endoribonuclease substrate; and
- (d) measuring cleavage of said substrate,

wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof.

2. A method for screening to identify an agent capable of modulating an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:

- (a) providing a nucleic acid sequence encoding said mRNA interferase or a functional fragment thereof;
- (a) expressing said nucleic acid sequence;
- (b) incubating the expressed nucleic acid sequence of step (b) with an endoribonuclease substrate under conditions capable of promoting endoribonuclease activity;
- (d) adding at least one agent to determine if it is capable of modulating endoribonuclease activity of said mRNA interferase or functional fragment thereof; and
- (e) measuring cleavage of said substrate,

wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof, and wherein a change in an amount of cleaved substrate in the presence of the at least one agent

identifies an agent capable of modulating an activity of said mRNA interferase or functional fragment thereof.

3. The method of claim 2, wherein said method is performed *in vitro* or in a cell.
4. The method of claim 2, wherein an agent capable of modulating an endoribonuclease activity of said mRNA interferase or a functional fragment thereof effectuates an increase in substrate cleavage.
5. The method of claim 2, wherein an agent capable of modulating an endoribonuclease activity of said mRNA interferase or a functional fragment thereof effectuates a decrease in substrate cleavage.
6. A method for modulating an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:
 - (c) providing a nucleic acid sequence encoding said mRNA interferase or a functional fragment thereof;
 - (b) expressing said nucleic acid sequence;
 - (c) incubating the expressed nucleic acid sequence of step (b) with an endoribonuclease substrate under conditions capable of promoting endoribonuclease activity;
 - (d) adding an agent of claim 2, said agent capable of modulating the endoribonuclease activity of said mRNA interferase or functional fragment thereof; and
 - (e) measuring cleavage of said substrate,

wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof, and wherein a change in an amount of cleaved substrate in the presence of the agent provides means to modulate endoribonuclease activity of said mRNA interferase or functional fragment thereof.

7. A method for detecting an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:

- (a) providing an amino acid sequence of said mRNA interferase;
- (b) incubating the amino acid sequence of step (a) with an endoribonuclease substrate under conditions capable of promoting endoribonuclease activity; and
- (c) measuring cleavage of said substrate,

wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof.

8. A method for screening to identify an agent capable of modulating an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:

- (a) providing an amino acid sequence of said mRNA interferase;
- (b) incubating the amino acid sequence of step (a) with an endoribonuclease substrate under conditions capable of promoting endoribonuclease activity;
- (c) adding at least one agent to determine if it is capable of modulating endoribonuclease activity of said mRNA interferase or functional fragment thereof; and
- (d) measuring the cleavage of said substrate,

wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof, and wherein a change in an amount of cleaved substrate in the presence of the at least one agent identifies an agent capable of modulating an activity of said mRNA interferase or functional fragment thereof.

9. The method of claim 8, wherein the method is performed *in vitro* or in a cell.

10. The method of claim 8, wherein an agent capable of modulating an endoribonuclease activity of said mRNA interferase or a functional fragment thereof effectuates an increase in substrate cleavage.

11. The method of claim 8, wherein an agent capable of modulating an endoribonuclease activity of said mRNA interferase or a functional fragment thereof effectuates a decrease in substrate cleavage.
12. A method for modulating an activity of an mRNA interferase or functional fragment thereof, wherein said activity is endoribonuclease activity, said method comprising:
 - (a) providing an amino acid sequence of said mRNA interferase;
 - (b) incubating the amino acid sequence of step (a) with an endoribonuclease substrate under conditions capable of promoting endoribonuclease activity;
 - (c) adding an agent of claim 8, said agent capable of modulating the endoribonuclease activity of said mRNA interferase or functional fragment thereof; and
 - (d) measuring cleavage of said substrate,wherein cleavage of said substrate indicates endoribonuclease activity and provides means to detect endoribonuclease activity of said mRNA interferase or a functional fragment thereof, and wherein a change in an amount of cleaved substrate in the presence of the agent provides means to modulate endoribonuclease activity of said mRNA interferase or functional fragment thereof.
13. A method for treating a patient with a disorder, said method comprising administering to said patient a therapeutically effective amount of a composition comprising at least one nucleic acid sequence encoding an mRNA interferase or a functional fragment thereof, and a pharmaceutically acceptable buffer, to alleviate symptoms of said disorder.
14. The method of claim 13, wherein expression of at least one nucleic acid sequence of said composition effectuates an increase in endoribonuclease substrate cleavage, to alleviate symptoms of said disorder.
15. The method of claim 14, wherein said disorder is a bacterial infection and said administering said therapeutically effective amount of a composition to said patient alleviates symptoms of the bacterial infection by reducing the number of bacteria in said patient.

16. The method of claim 15, wherein said bacterial infection comprises at least one antibiotic resistant bacterial strain.
17. The method of claim 14, wherein said disorder is a hyperproliferative disorder and said administering said therapeutically effective amount of a composition to said patient alleviates symptoms of said hyperproliferative disorder by reducing the number of hyperproliferative disorder cells in said patient.
18. A method for treating a patient with a disorder, said method comprising administering to said patient a therapeutically effective amount of a composition comprising an mRNA interferase or functional fragment thereof and a pharmaceutically acceptable buffer, to alleviate symptoms of said disorder.
19. The method of claim 18, wherein the mRNA interferase or functional fragment thereof of said composition effectuates an increase in endoribonuclease substrate cleavage, to alleviate symptoms of said disorder.
20. The method of claim 19, wherein said disorder is a bacterial infection and said administering said therapeutically effective amount of a composition to said patient alleviates symptoms of the bacterial infection by reducing the number of bacteria in said patient.
21. The method of claim 20, wherein said bacterial infection comprises at least one antibiotic resistant bacterial strain.
22. The method of claim 19, wherein said disorder is a hyperproliferative disorder and said administering said therapeutically effective amount of a composition to said patient alleviates symptoms of the hyperproliferative disorder by reducing the number of hyperproliferative cells in said patient.
23. The method of either of claims 17 or 22, wherein said hyperproliferative disorder is selected from the group consisting of dysplasias and metaplasias of different tissues, inflammatory

conditions, autoimmune diseases, hyperproliferative skin disorders, psoriasis, allergy/asthma, atherosclerosis, and restenosis after angioplastic surgery.

24. A method for making a polypeptide in a cell, said method comprising:

(a) transfecting said cell with a nucleic acid sequence encoding said polypeptide, wherein the nucleic acid sequence encoding said polypeptide is mutated to replace mRNA interferase recognition sequences with an alternate triplet codon, wherein amino acid sequences of said polypeptide encoded by said mutated nucleic acid sequence are not altered by said mutating;

(b) transfecting said cell with a nucleic acid sequence encoding an mRNA interferase, wherein said mRNA interferase recognizes said mRNA interferase recognition sequences; and

(c) expressing the nucleic acid sequences of step (a) and (b) in said cell, wherein expressing the nucleic acid sequences of step (a) and (b) in said cell provides means to produce the polypeptide in said cell.

25. The method of claim 24, wherein the mRNA recognition sequence is an Adenine-Cytosine-Adenine (ACA) sequence and the mRNA interferase is MazF comprising SEQ ID NO: 2 or a functional fragment thereof.

26. The method of claim 24, wherein the mRNA recognition sequence is a Uracil-Adenine-X (UAX) sequence, wherein X is a Cytosine (C), A, or U, and the mRNA interferase is PemK comprising SEQ ID NO: 4 or a functional fragment thereof.

27. The method of claim 25, wherein expression of a nucleic acid of step (b) reduces or inhibits synthesis of cellular polypeptides encoded by nucleic acid sequences comprising ACA sequences.

28. The method of claim 26, wherein expression of a nucleic acid of step (b) reduces or inhibits synthesis of cellular polypeptides encoded by nucleic acid sequences comprising UAX sequences.

29. The method of claim 24, wherein step (a) and step (b) are performed simultaneously.
30. The method of claim 24, further comprising incubating said cell prior to or during step (c) in media comprising at least one radioactively labeled isotope.
31. A method for making a polypeptide, said method comprising:
 - (a) providing a nucleic acid sequence encoding said polypeptide, wherein the nucleic acid sequence encoding said polypeptide is mutated to replace mRNA interferase recognition sequences with an alternate triplet codon, wherein amino acid sequences of said polypeptide encoded by said mutated nucleic acid sequence are not altered by said mutating;
 - (b) providing a nucleic acid sequence encoding an mRNA interferase, wherein said mRNA interferase recognizes said mRNA interferase recognition sequences; and
 - (c) expressing the nucleic acid sequences of step (a) and (b), wherein expressing the nucleic acid sequences of step (a) and (b) provides means to produce the polypeptide.
32. The method of claim 31, wherein the mRNA recognition sequence is an ACA sequence and the mRNA interferase is MazF comprising SEQ ID NO: 2 or a functional fragment thereof; or wherein the mRNA recognition sequence is a UAX sequence, wherein X is a C, A, or U, and the mRNA interferase is PemK comprising SEQ ID NO: 4 or a functional fragment thereof.
33. A method for making a plurality of polyribonucleotide sequences, said method comprising:
 - (a) providing a first and a second nucleic acid sequence, wherein a region of said first nucleic acid sequence is complementary to a region of said second nucleic acid sequence and neither complementary region of said first or second nucleic acid sequence comprises a sequence complementary to an mRNA interferase recognition site, and each of said first and second nucleic acid sequences is phosphorylated at its 5' terminus;
 - (b) annealing said first and second nucleic acid sequences via a complementary region of said first and second nucleic acid sequences to form a double

stranded nucleic acid sequence comprising a complementary region flanked by single stranded overhangs, wherein each of said single stranded overhangs comprises at least one sequence complementary to an mRNA interferase recognition site and said single stranded overhangs are complementary to each other;

- (c) ligating annealed first and second nucleic acid sequences via complementary single stranded overhangs to form a concatamer comprising a plurality of tandem repeats of annealed first and second nucleic acid sequences;
- (d) amplifying said concatamer using a first primer comprising a T7 promoter and a region complementary to said first nucleic acid sequence and a second primer complementary to said second nucleic acid sequence, wherein said amplifying produces a plurality of concatamers comprising a T7 promoter;
- (e) transcribing RNA molecules from said plurality of concatamers using T7 RNA polymerase, wherein each of said RNA molecules comprises a plurality of tandem repeats of a polyribonucleotide sequence flanked by mRNA interferase recognition sites; and
- (f) digesting said RNA molecules with an mRNA interferase capable of cleaving RNA at said interferase recognition sites, wherein said digesting produces a plurality of said polyribonucleotide sequences.

34. The method of claim 33, wherein the mRNA recognition sequence is an ACA sequence and the mRNA interferase is MazF comprising SEQ ID NO: 2 or a functional fragment thereof; or wherein the mRNA recognition sequence is a UAX sequence, wherein X is a C, A, or U, and the mRNA interferase is PemK comprising SEQ ID NO: 4 or a functional fragment thereof.

35. An isolated nucleic acid sequence which encodes a polypeptide having sequence and/or structural homology to an mRNA interferase or a functional fragment thereof, wherein said polypeptide is capable of exhibiting endoribonuclease activity; an expression vector comprising said isolated nucleic acid sequence and a cell comprising said expression vector; an expression vector comprising said isolated nucleic acid sequence, wherein said nucleic acid sequence is operably linked to a regulatory sequence, and a cell comprising said expression vector; a

transgenic animal comprising said isolated nucleic acid sequence, wherein the nucleic acid sequence is expressed in at least one cell of the transgenic animal; an isolated amino acid sequence comprising a polypeptide having sequence and/or structural homology to an mRNA interferase or a functional fragment thereof, wherein said polypeptide is capable of exhibiting endoribonuclease activity; an expression vector encoding said polypeptide and a cell comprising said expression vector; an expression vector encoding said polypeptide, wherein expression of said polypeptide is under the control of a regulatory sequence, and a cell comprising said expression vector; a transgenic animal expressing said polypeptide, wherein the polypeptide is expressed in at least one cell of the transgenic animal; a composition comprising either said isolated nucleic acid sequence or said polypeptide and a pharmaceutically acceptable buffer and use of either of said compositions for the treatment of a patient with a disorder to alleviate symptoms of said disorder; and a kit comprising said isolated nucleic acid sequence, an isolated amino acid sequence comprising said polypeptide or a functional fragment thereof, an mRNA interferase activity compatible buffer, and instructional materials.